Title

Consequences of cytotoxic T lymphocyte interaction with major histocompatibility complex class I-expressing neurons in vivo.

Journal

The Journal of experimental medicine, 182(5)

ISSN

0022-1007

Authors

Rall, GF
Mucke, L
Oldstone, MB

Publication Date

1995-11-01

DOI

10.1084/jem.182.5.1201

Peer reviewed

Consequences of Cytotoxic T Lymphocyte Interaction with Major Histocompatibility Complex Class I–expressing Neurons In Vivo

By Glenn F. Rall, Lennart Mucke, and Michael B. A. Oldstone

From The Scripps Research Institute, Division of Virology, Department of Neuropharmacology, La Jolla, California 92037

Summary

Neurons have evolved strategies to evade immune surveillance that include an inability to synthesize the heavy chain of the class I major histocompatibility complex (MHC), proteins that are necessary for cytotoxic T lymphocyte (CTL) recognition of target cells. Multiple viruses have taken advantage of the lack of CTL-mediated recognition and killing of neurons by establishing persistent neuronal infections and thereby escaping attack by antiviral CTL. We have expressed a class I MHC molecule (D^b) in neurons of transgenic mice using the neuron-specific enolase (NSE) promoter to determine the pathogenic consequences of CTL recognition of virally infected, MHC-expressing central nervous system (CNS) neurons. The NSE-D^b transgene was expressed in H-2^b founder mice, and transgene-derived messenger RNA was detected by reverse transcriptase–polymerase chain reaction in transgenic brains from several lines. Purified primary neurons from transgenic but not from nontransgenic mice adhered to coverslips coated with a conformation-dependent monoclonal antibody directed against the D^b molecule and presented viral peptide to CTL in an MHC-restricted manner, indicating that the D^b molecule was expressed on transgenic neurons in a functional form. Transgenic mice infected with the neurotropic lymphocytic choriomeningitis virus (LCMV) and given anti-LCMV, MHC-restricted CTL displayed a high morbidity and mortality when compared with controls receiving MHC-mismatched CTL or expressing alternative transgenes. After CTL transfer, transgenic brains showed an increased number of $CD8^+$ cells compared with nontransgenic controls as well as an increased rate of clearance of infectious virus from the CNS. Additionally, an increase in blood–brain barrier permeability was detected during viral clearance in NSE-D^b transgenic mice and lasted several months after clearance of virus from neurons. In contrast, LCMV-infected, nontransgenic littermates and mice expressing other gene products from the NSE promoter showed no CNS disease, no increased intraparenchymal CTL, and no blood–brain barrier damage after the adoptive transfer of antiviral CTL. Our study indicates that viral infections and CTL–CNS interactions may induce blood–brain barrier disruptions and neurologic disease by a "hit-and-run" mechanism, triggering a cascade of pathogenic events that proceeds in the absence of continual viral stimulation.

Several properties unique to the central nervous system (CNS)[1] led to the concept that the CNS is an immune-privileged site (1, 2). To recognize and lyse target cells within the brain parenchyma, immune effector cells must cross the blood–brain barrier, a tightly packed wall of endothelia that separates the circulatory system from CNS cells. While nutrients and oxygen either diffuse or are transported across the barrier, the passage of blood-borne proteins and cells (including red blood cells and resting lymphocytes) into the brain is restricted, because of both the tight junctions between the capillary endothelia and a lack of active cell transport by the endothelial cells themselves (for review see references 3 and 4). However, the barrier does not isolate the parenchyma from activated immune cells; activated T lymphocytes and monocyte/macrophages traverse the intact blood–brain barrier and traffic through the brain (5–9). These infiltrating, activated T cells are restricted from recognizing CNS targets, however, at least partly because of a paucity of cell surface molecules required for T cell–target interaction. Resident cells of the CNS normally express low to undetectable levels of both

[1] *Abbreviations used in this paper:* aa, amino acid; CNS, central nervous system; LCMV, lymphocytic choriomeningitis virus; mRNA, messenger RNA; NP, nucleoprotein; NSE, neuron-specific enolase.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/11/1201/12 $2.00
Volume 182 November 1995 1201–1212

class I and II MHC molecules (10–12). The class I MHC glycoproteins present foreign (viral) proteins in the form of 8–11 amino acid (aa) peptides to the T cell receptor of $CD8^+$ cytotoxic T lymphocytes. Neurons, both in vivo (13–15) and in vitro (12, 14, 16–18), do not express MHC class I molecules and, hence, do not serve as targets for CTL-mediated lysis. Multiple RNA and DNA viruses, including members of the *Herpes-*, *Paramyxo-*, *Rhabdo-*, *Picorna-*, and *Arenavirus* families, infect neurons of the CNS (for review see references 19–21). Viruses from each of these families are able to establish latent or persistent infections in CNS neurons, most likely because viral persistence in neurons through immune evasion is preferable to lysis by antiviral CTL of these essential and irreplaceable cells.

Earlier studies began to characterize neuron–virus–CTL interactions using the OBL-21 neuronal cell line (14). These cells contain neurofilaments and show electrophysiologic responses consistent with neurons (22), although, unlike CNS neurons, they are dividing cells. Like neurons in vivo, OBL-21 cells fail to express MHC molecules, and, although infected with virus and expressing viral proteins on their surfaces, were not lysed by antiviral CTL (14). However, after complementation of class I MHC antigen–presenting capacity by transfection with a D^b-encoding retroviral vector, infected OBL-21 cells were lysed by antiviral CTL (14).

In this paper we extend these in vitro observations in vivo. First, transgenic mice that express a class I MHC molecule on CNS neurons were established. Second, MHC-expressing neurons purified from such transgenic mice were susceptible to lysis by virus-specific MHC-restricted CTL, whereas neurons from nontransgenic mice were not. CNS disease was induced in virally infected transgenic mice after adoptive transfer of virus-specific, MHC-restricted CTL. The disease lasted for several months after CTL transfer and was associated with an increased number of intraparenchymal $CD8^+$ cells and a chronic destabilization of the blood–brain barrier.

Materials and Methods

Animals, Cells, and Viruses. C57BL/6 ($H\text{-}2^b$), SJL ($H\text{-}2^s$), and BALB/cByJ ($H\text{-}2^d$) inbred mice were obtained from the closed breeding facility of The Scripps Research Institute (La Jolla, CA). Animals were maintained in conditions consistent with American Association for the Accreditation of Laboratory Animal Care regulations throughout the course of the investigation.

Lymphocytic choriomeningitis virus (LCMV) strain Armstrong CA 1371 clone 53b (LCMV ARM) was used in these studies. The origin, cloning and plaque purification of stock virus have been reported (23, 24). Virus was grown in baby hamster kidney cells and was plaque purified and titered on Vero cell monolayers as described (24). BALB clone 7 ($H\text{-}2^d$) fibroblasts and Vero cells were grown in MEM supplemented with 10% heat-inactivated FCS, 1 mM L-glutamine, 10^4 U/ml penicillin, and 10 mg/ml streptomycin. MC57 ($H\text{-}2^b$) fibroblasts were cultured in RPMI 1640 with the supplements listed above. CTL clones restricted by $H\text{-}2^b$ (clone NP18 recognizing nucleoprotein [NP] aa residues 396–404; 25) and $H\text{-}2^d$ (clone HD8 recognizing NP aa residues 118–127; 26) were used. These clones were maintained in culture as described (25).

Persistent infection of mice was achieved by inoculation of 1,000 PFU of LCMV ARM intracerebrally into newborn mice within 18 h of birth. Such mice carry infectious virus and viral nucleic acid in many cells, including neurons, throughout their lives (27).

Cloning Procedures and Establishment of Transgenic Mice. Standard molecular techniques (28, 29) were used for the construction of the NSE-D^b transgene. The NSE expression vector (30, 31) was obtained from S. Forss-Petter, (The Scripps Research Institute) and the Mo/D^b clone (32) from R. Flavell, (Yale University, New Haven, CT). Promoter–transgene junctions created by the subcloning were sequenced before microinjection. Fertilized oocytes were obtained from (C57Bl/6 × SJL) female mice. Purification of the transgene, preparation of mice, microinjection, and embryo implantations were carried out as described (33–35).

Transgenic mice were identified by tail biopsy and subsequent DNA isolation. 10 μg of DNA was slot blotted onto filters (Nytran; Schleicher & Schuell, Inc., Keene, NH) and probed with the SV40 sequence located at the 3′ end of the NSE expression cassette.

Reverse Transcriptase–PCR (RT-PCR). Brains and other organs were removed from saline-perfused mice and were snap frozen in liquid nitrogen. The tissues were stored at −70°C before homogenization. Tissues were homogenized using a Virtishear (Virtis Co., Inc., Gardiner, NY) for 30 s in 1 ml Tri-reagent/100 mg tissue (Molecular Research Center, Inc., Cincinnati, OH), and RNA was purified according to their recommended protocol. 500 ng of total RNA was reverse transcribed by the random priming method, using random hexamers (Pharmacia Biotech, Inc., Piscataway, NJ) and reverse transcriptase (GIBCO BRL, Gaithersburg, MD). The RNA–cDNA duplex was then subjected to 40 rounds of PCR (60°C annealing temperature; Taq I polymerase from GIBCO BRL), using 20-mer oligonucleotide primers designed to amplify transgene specific sequences: A = 5′ GAGAT CGACT CTAGA GGATC 3′; B = 5′ GCGCT CTGGT TGTAG TAGCC 3′. The PCR products were visualized by ethidium bromide intercalation on a 1% agarose gel.

Hippocampal Neuron Cultures and Protein Detection. Embryonic mice (day 16–18) were used for the preparation of dissociated hippocampal neuron cultures following the protocol of Banker and Goslin (36) with minor modifications (37; Rall, G. F., unpublished results). Briefly, hippocampi were dissected from embryos and placed in ice-cold plating medium (DMEM supplemented with 10% fetal bovine serum, 10^4 U/ml penicillin, and 10 mg/ml streptomycin). The tissues were washed three times with ice-cold PBS and digested with trypsin-EDTA (Sigma Chemical Co., St. Louis, MO) at 37°C for 15 min. The tissue was washed twice with plating medium, mechanically dissociated with fire-polished pipettes, and passed through a cell strainer (Falcon Labware, Oxnard, CA) to obtain a single-cell suspension. The neurons were spun at 150 *g* for 7 min onto a serum cushion, resuspended, counted, and plated onto either poly-L-lysine (Sigma Chemical Co.) or antibody-coated, acid-washed, round glass coverslips (Carolina Biological Supply Co., Burlington, NC) at a density of 500 cells/mm^2.

Individual coverslips were incubated with affinity-purified goat anti–mouse IgG (Cappel Laboratories, Cochranville, PA; 10 μg/slip) for 4 h, washed with PBS and then coated with mAbs specific for either the D^b class I MHC molecule (B22. 249.R1, 1:500; Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) or to the L^d class I MHC molecule (30-5-7S, 1:500; Cedarlane Laborato-

ries Ltd.) in doses of 3 μg/coverslip (38, 39). Coverslips were placed in a humidified incubator overnight, and, immediately before neuron plating, the antibody was aspirated and the slips were washed once with PBS, pH 7.4.

4 h after the neurons were added to the coverslips, the slips were placed on top of a confluent primary astrocyte feeder layer (40), in serum-free neurobasal medium (GIBCO BRL) supplemented with growth factors (B27 additive; GIBCO BRL). The cells were maintained at 37°C in 5% CO_2.

In Vitro Killing Assay. Neurons cultured in vitro for 48 h were either untreated or were coated with peptides representing LCMV CTL epitopes restricted by either H-2^b (NP aa 396–404: NH_2-FQPQNGQFI-COOH) or H-2^d (NP aa 118–127: NH_2-SERPQASGVG-COOH) at a concentration of 40 μg/2 × 10^4 targets 1 h before the addition of CTL. H-2^b– or H-2^d–restricted CTL clones were then added to the cultures at a 1:1 ratio, and they were allowed to incubate for 6 h. After removal of the clones and debris by successive PBS washes, the neurons were fixed with 2% paraformaldehyde and were counterstained with hematoxylin. 10 random fields were counted per slide, and the results were confirmed in three independent experiments. MC57 (H-2^b) and BALB cl7 (H-2^d) fibroblasts served as controls.

Adoptive Transfer of Memory CTL into Persistently Infected Mice. Adoptive transfer of antiviral CTL into persistently infected mice has been described (41). The memory cytotoxic effector cells transferred were obtained from normal adult C57Bl/6 or SJL mice infected 60–120 d earlier by intraperitoneal inoculation of 2 × 10^5 PFU of LCMV. Spleens were removed and dissociated, and a single-cell suspension was prepared and cultured in 24-well plates with syngeneic LCMV-infected and irradiated peritoneal macrophages (as stimulators) and syngeneic irradiated spleen cells (as feeder cells). Adherent lymphocytes (secondaries) were expanded three times on infected, irradiated stimulator cells before transfer into age-matched (6–13-wk-old) persistently infected mice and nontransgenic controls.

Transgenic and nontransgenic mice (H-2^b) were bred with nontransgenic SJL mice (H-2^s) to generate H-2^{bxs} mice for the adoptive transfer experiments. The progeny of such breedings was infected with LCMV (10^3 PFU) within 24 h of birth to establish persistent infections. The presence of virus in these mice (between 4 and 8 wk) was determined before the adoptive transfer by serum plaque assay. 24 h before the transfer of 5 × 10^6 secondary CTL intraperitoneally, mice were irradiated with 400 rad to accommodate the adoptively transferred cells. The health of the animals was monitored daily from 1 to 30 d after adoptive transfer and weekly thereafter, and evaluated on a four-level scale: 1, healthy and indistinguishable from uninfected normal mice; 2, fur slightly ruffled, posture hunched, eyes partially closed, reduced mobility; 3, ruffled, hunched, and tremulous, eyes mucous filled and closed, significant motor impairment; 4, dead.

Viral titers in serum were monitored 6, 15, 60, and 120 d after adoptive transfer, and mice from experimental and control groups were killed at 15 and 60 d after adoptive transfer for histological studies.

Detection of CD8$^+$ Lymphocytes within Brain Sections. Mice were deeply anesthetized with a 3.8% solution of chloral hydrate and were perfused with normal saline. Brains were rapidly removed, quick frozen in a dry ice/isopentane bath, and stored at −70°C. 10-μm horizontal sections were cut and fixed in 95% ethanol for 15 min at −20°C, were blocked with avidin-biotin (Vector Laboratories, Inc., Burlingame, CA), and were incubated for 1.5 h with mAbs to the murine CD8 molecule (CD8a and Ly3, diluted 1:50; Pharmingen, San Diego, CA). After washing, sections were incubated with biotinylated secondary antibodies, followed by an avidin-biotin reaction (ABC Elite; Vector Laboratories, Inc.) and a color reaction using diaminobenzidene and hydrogen peroxide. Sections were lightly counterstained with hematoxylin and were mounted with Aquamount (Lerner Laboratories, New Haven, CT).

The median values of the number of CD8$^+$ lymphocytes per brain section within a given group were calculated. At least four horizontal sections were evaluated per mouse brain.

Blood–Brain Barrier Permeability. Mice were deeply anesthetized with a 3.8% solution of chloral hydrate. 2 ml of a 2% Evans blue solution in PBS was infused transcardially over a 1-min period. 3 min later, mice were perfused with 4% paraformaldehyde in PBS, and brains were removed and stored in 0.2% paraformaldehyde in PBS.

Results

The Class I MHC Molecule, D^b, Is Expressed in Neurons of Transgenic Mice. Transgenic mice with neuronal expression of the class I MHC molecule D^b were established by injection of oocytes with a minigene containing the complete D^b coding sequence under the control of the NSE promoter (Fig. 1). The D^b minigene (40) was cloned into the BamHI restriction site within exon 2 of the NSE cassette (Fig. 1), and the resulting plasmid was characterized by restriction analysis and sequencing of promoter–transgene junctions. The linearized, gel-purified NSE-D^b transgene was then microinjected into ~200 (C57Bl/6 × SJL)$_{F2}$ one–cell stage embryos.

From this microinjection, 42 mice were born, 12 of which contained the transgene. Five of these founder mice were selected for breeding and were back-crossed onto the C57Bl/6 background. After three back-crosses, the CTL response within these mice was completely restricted to the H-2^b haplotype as determined by chromium release assays (data not shown).

Tissue expression of the NSE-D^b messenger RNA (mRNA) was analyzed by RT-PCR on RNA isolated from transgenic and nontransgenic mouse tissues (brain,

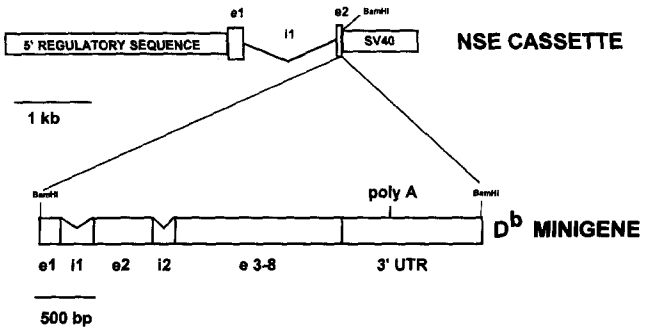

Figure 1. Structure of the NSE-D^b fusion gene. The NSE-D^b construct consists of 2.8 kb of 5′ NSE regulatory sequence, NSE exon 1 and intron 1, and 15 bp of NSE exon 2 plus polylinker. The D^b minigene includes all 8 exons and the first two introns of the murine D^b gene. Polyadenylation signals are provided by 3′ untranslated sequences (UTR) of the D^b minigene. SV40 sequence at the 3′ end of the construct facilitates identification of transgenic mice by slot blot analysis of genomic DNA. *e*, exon; *i*, intron.

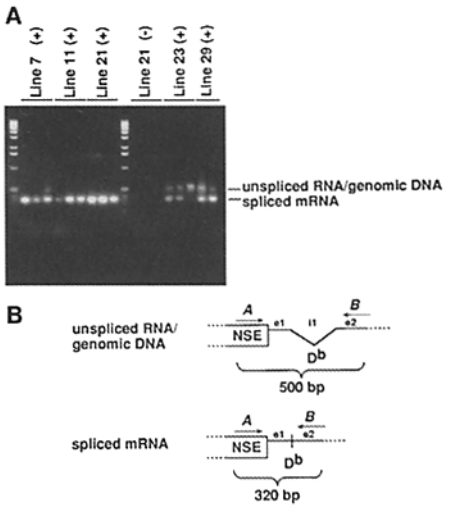

Figure 2. Expression of D^b mRNA in brains of transgenic mice. Brains were removed from transgenic (+) and nontransgenic (−) mice. Total RNA was extracted and subjected to RT-PCR as described in Materials and Methods. Primers *A* and *B* were used to amplify transgene-specific mRNA. The primer pair was designed to differentiate spliced and unspliced templates by size; the primers span intron 1 of the D^b sequence, so that the predicted PCR product derived from spliced mRNA is 180 bp smaller than the product derived from either unspliced RNA or residual DNA in the preparation (Fig. 2 *B*). Spliced mRNA (as a 320-bp PCR product) was detected in each of the five transgenic lines, and, in some cases, a PCR product corresponding to DNA or unspliced RNA (500 bp) was seen (lines 23 and 29). No PCR product was amplified in nontransgenic mice (line 21−). Results obtained in three mice per group are shown.

spleen, kidney, pancreas, liver, testes, gut, thymus, lung, heart, and optic and sciatic nerves). Total brain RNA isolated from mice of six transgenic lines (7, 9, 11, 21, 23, and 29) contained transgene-derived mRNA, whereas brain RNA from nontransgenic littermates did not (Fig. 2 *A*). Transgenic lines 9 (data not shown) and 11 were used for subsequent experiments. No spliced NSE-D^b mRNA was detected in other organs except for in the testes of line 29 (data not shown). Line 29 was not used for further experiments.

Total RNA purified from primary astrocyte/microglial cultures derived from transgenic neonatal mice of lines 9 and 11 were also analyzed by the RT-PCR approach. No RNA PCR product corresponding to the NSE-D^b transgene was detected in these cultures (data not shown).

The NSE-D^b expression at the protein level was too low to be detected by immunostaining of tissue sections. However, neuronal expression of D^b protein was demonstrated by culturing primary embryonic hippocampal neurons and testing their ability to grow on glass coverslips coated with an mAb specific for either D^b or for a heterologous MHC molecule (anti-L^d). Neurons from transgenic (D^b-expressing) mice adhered to anti-D^b–, but not to anti-L^d–coated coverslips (Fig. 3). Nontransgenic neurons did not attach to either type of coverslip, confirming the absence of MHC class I on their surface. Notably, the antibody used, B.22.249, is conformation dependent (32). Therefore, the D^b molecule is expressed in a conformationally correct form on the surface of transgenic neurons.

The Transgene-derived D^b Molecule Conferred CTL Recognition and Lysability to CNS Neurons In Vitro. We next determined whether transgenic neurons could present antigen and serve as targets for CTL-mediated lysis in a neuronal survival assay. Primary neurons do not take up ^{51}Cr; therefore, instead of standard chromium release assays, an alternative assay was used. Transgenic and nontransgenic neurons were incubated with peptides encoding LCMV-specific epitopes that are restricted by either H-2^b or H-2^d (25, 26, 35). Peptide-coated neurons were reacted with specific anti-LCMV H-2^b– or H-2^d–restricted CTL clones, and the number of viable neurons was determined after a 6-h incubation. D^b-expressing neurons incubated with H-2^b–restricted viral peptide and a matched CTL clone showed a significant reduction (73%) in cell viability ($P < 0.05$), whereas nontransgenic neurons did not (Fig. 4). These data were reproducible in three independent experiments.

Persistently Infected, Transgenic Mice Developed CNS Disease after Adoptive Transfer of LCMV-specific D^b-restricted CTL. NSE-D^b–expressing mice were maintained in a specific pathogen-free facility. No differences in appearance, fecundity, or life span were noted between uninfected transgenic animals and nontransgenic littermate controls (data not shown).

To determine the antigen-presenting capacity of transgenic neurons in vivo and the consequences of their interaction with D^b-restricted CTL, mice were infected as neonates with LCMV. In this persistent infection, most tissues, including the CNS, liver, spleen, and kidney, contain LCMV; within the CNS, the neuron is the only cell type infected (42, 43). Adoptive transfer of virus-specific, MHC-restricted CTL (41, 44) results in CTL-mediated clearance of LCMV from all sites, other than neurons and renal glomeruli, within 10–30 d after transfer. The eventual elimination of virus and viral nucleic acid from neurons occurs over a longer period (60–120 d after adoptive transfer) and does not involve obvious infiltration of the brain parenchyma (41). The mechanism of viral clearance from nontransgenic, persistently infected brains is not completely understood, but does not seem to occur via classical CTL–target cell interactions.

NSE-D^b transgenic mice, NSE–amyloid precursor protein (APP) transgenic mice (expressing APP as a non-MHC transgenic control; 31), and nontransgenic littermates were infected with LCMV at birth. Each of these groups of mice was generated by breeding an H-2^b transgenic parent with a nontransgenic SJL (H-2^s) mouse, resulting in H-2^{bxs} offspring. Secondary immune T lymphocytes raised in H-2^b or H-2^s mice were then adoptively transferred into the persistently infected H-2^{bxs} animals. This experiment was based on the following rationale: both H-2^b and H-2^s anti-LCMV CTL could recognize virally infected cells in peripheral tissues of persistently infected H-2^{bxs} mice, but only H-2^b anti-LCMV CTL would recognize viral antigen complexed with the D^b molecule on NSE-D^b transgenic neurons. Thus, if mice became sick or died as a conse-

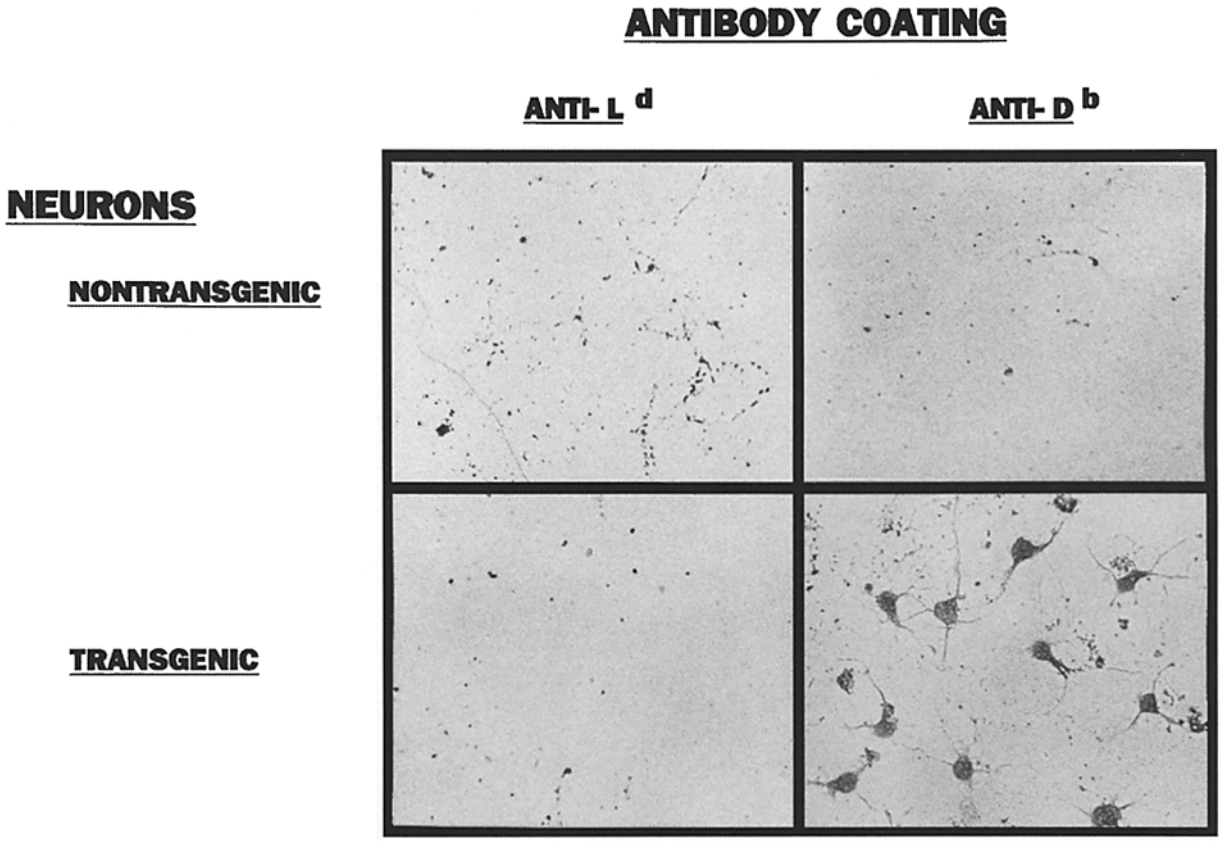

Figure 3. Expression of D^b molecules on the surface of neurons isolated from the CNS of transgenic mice. Primary embryonic hippocampal neurons from transgenic and nontransgenic litters were plated onto coverslips coated with mAbs to either D^b (B22.249) or L^d (30-5-7S) as described in Materials and Methods. Photographs were taken 48 h after plating. Staining with antibody against the neuronal marker, microtubule-associated protein 2 indicated that >95% of the adherent cells were neurons.

quence of CTL interactions with the D^b expressed on neurons, this should be seen only in NSE-D^b transgenic mice receiving the H-2^b–restricted CTL (Fig. 5).

Animal health was monitored daily and independently by at least two investigators after adoptive transfer of CTL. The genetic profile of the mice and the CTL that they received were unknown to the observers to avoid bias. Persistently infected mice appeared healthy before the adoptive transfer. 5–15 d after transfer, when CTL are killing virally infected MHC-expressing cells in peripheral tissues (liver, spleen, kidney, etc.) (41), some mice showed mild to moderate illness (clinical levels 2–3) in all experimental groups. The combined results (Table 1) of three independent adoptive transfer experiments using a minimum of four mice per experimental group revealed the following: (*a*) NSE-D^b transgenic mice exposed to H-2^b CTL showed significantly higher morbidity and mortality than NSE-APP or nontransgenic controls receiving similar immunotherapy; (*b*) NSE-D^b mice had a higher morbidity and mortality when given H-2^b CTL compared with H-2^s CTL, whereas H-2^b and H-2^s CTL had a similar effect in the control groups; and (*c*) NSE-D^b mice that survived the initial 15-d viral clearance period remained sick throughout the observation period (clinical stages 2–3), whereas control mice returned to clinical stage 1 after viral clearance.

NSE-D^b Transgenic Mice Receiving Adoptive CTL Therapy Showed Increased Numbers of $CD8^+$ Lymphocytes in the CNS Parenchyma and Accelerated Viral Clearance. Brains were removed from mice at various time points after adoptive transfer and stained with mAbs to murine CD8 to compare the number of infiltrating CTL within the brains of NSE-D^b transgenic versus control mice. Photomicrographs of representative murine brains are shown in Fig. 6, and a quantitative summary is shown in Table 2. Uninfected NSE-D^b transgenic brains contained rare CTL (Fig. 6 *A*; Table 2, group A), suggesting that the expression of D^b on neurons per se is not sufficient to recruit T cells into the parenchyma. The $CD8^+$ CTL found within the brains of persistently infected mice were distributed uniformly throughout the parenchyma, and both transgenic and nontransgenic mice killed at various ages after neonatal LCMV infection contained similar numbers of intraparenchymal $CD8^+$ T cells (Fig. 6 *B*; Table 2, groups B and C). In contrast, 15 d after adoptive transfer of D^b-restricted anti-LCMV CTL, there was a marked increase in the number of $CD8^+$ T cells within brains of persistently infected, NSE-D^b trans-

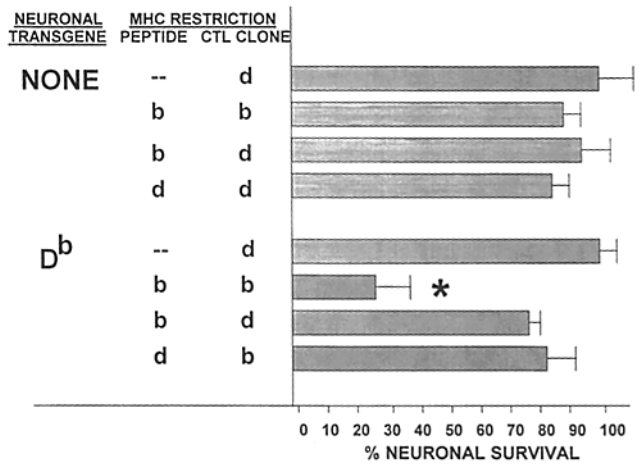

Figure 4. Lysis of D^b-expressing primary neurons coated with appropriate viral peptide by D^b-restricted LCMV-specific CTL. Primary neurons were incubated for 1 h with peptides encoding epitopes for LCMV restricted by either H-2^b (viral NP, aa 396–404) or H-2^d (NP, aa 118–127) or with no peptide (- -). One hour after peptide addition, H-2^b– or H-2^d–restricted antiviral CTL were added to the cultures. 5–6 h later, neurons were fixed, stained, and counted as described in Materials and Methods. Numbers of neurons in cultures incubated with no peptide and H-2^d CTL were arbitrarily defined as 100%. Data points represent the number of remaining neurons expressed as a percentage of this control; error bars indicate SD. Similar results were obtained independently in two additional experiments (data not shown). * P values <0.05 by the Student's t test.

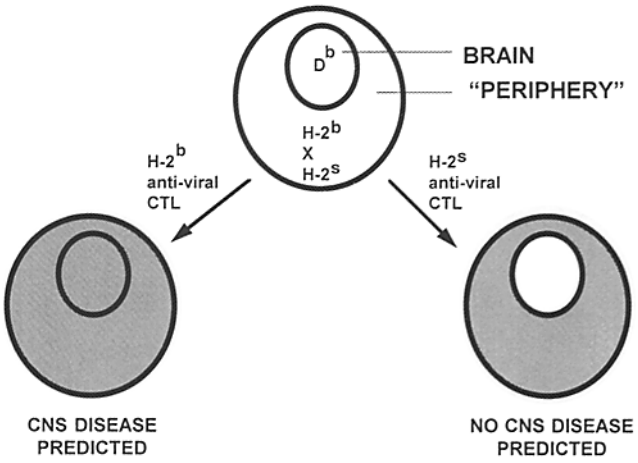

Figure 5. Rationale of the adoptive transfer protocol. See text for description.

genic mice (Fig. 6 *C*; Table 2, group D), which was not seen in nontransgenic controls receiving similar immunotherapy (Fig. 6 *D*; Table 2, group E). Interestingly, in areas densely populated with LCMV-infected neurons, such as the hippocampus and the Purkinje cell layer of the cerebellum, no obvious signs of neuronal dropout were seen, suggesting that infected neurons were not lysed by CTL. By day 40, the number of CTL in the brains of transgenic mice had decreased (Fig. 6 *E*; Table 2, group F). Similar findings were obtained in at least three mice per experimental group.

Brain tissues taken from mice 15 and 40 d after adoptive transfer were also plaque assayed to assess the effect of the neuronal MHC expression on the clearance of virus from transgenic brains. While virus was still present in brains of nontransgenic control mice 15 d after adoptive transfer, virus was no longer detectable at this time point in transgenic brains receiving the H-2^b secondary lymphocytes (Fig. 7). Notably, despite an accelerated viral clearance, transgenic mice remained sick for up to 120 d after the adoptive transfer.

The Increased Sickness of NSE-D^b Transgenic Mice Correlated with a Chronic Disruption of the Blood–Brain Barrier. To determine whether the increased number of CTL in the CNS

Table 1. *Occurrence of Sickness and Death in NSE-D^b, NSE-APP, and Nontransgenic Persistently Infected Mice Receiving H-2^b– or H-2^s–restricted Antiviral CTL**

			Injury after adoptive transfer in days (in %)					
			Day 5–15		Day 15–60		Day 60–120	
Transgene	Mice	CTL MHC	Sickness	Death	Sickness	Death	Sickness	Death
	n							
NSE-D^b, line 9	18	H-2^b	100	33	85	0	69	0
NSE-D^b, line 9	8	H-2^s	50	12	14	0	0	0
NSE-D^b, line 11	14	H-2^b	93	43	78	0	88	0
NSE-D^b, line 11	10	H-2^s	25	0	11	0	11	0
NSE-APP and	52	H-2^b	40	8	4	0	10	6
Nontransgenic	15	H-2^s	47	7	12	0	14	0

*5 × 10^6 CTL were transferred into persistently infected H-2^{bxs} NSE-D^b, NSE-APP, or nontransgenic mice. Health was monitored daily, and accumulated sickness and death measurements are presented as the percentage of the total number of animals in each group.

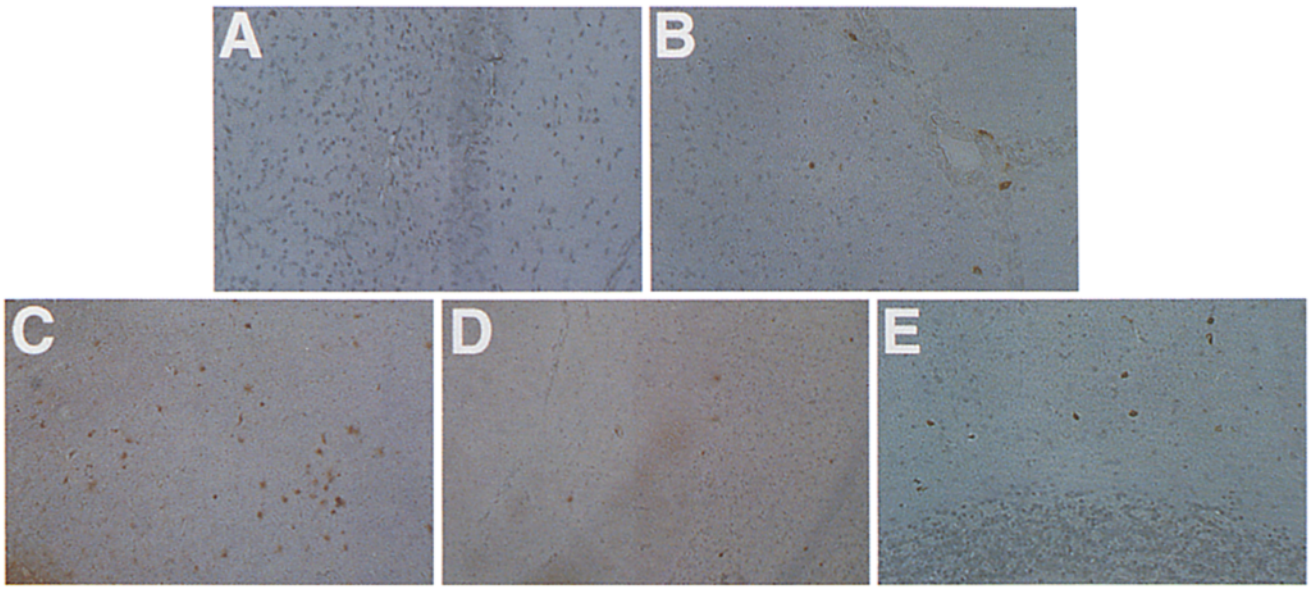

Figure 6. Increased number of $CD8^+$ cells in brains of transgenic, persistently infected mice after adoptive transfer of H-2^b–restricted anti-LCMV CTL. Sections of caudate (*A–D*) or cerebellum (*E*) were stained for the presence of $CD8^+$ lymphocytes as described in Materials and Methods, and were counterstained with hematoxylin. (*A*) Uninfected NSE-D^b transgenic mouse, no CTL given; (*B*) persistently infected NSE-D^b mouse, no CTL given; (*C*) persistently infected, NSE-D^b transgenic mouse, day 15 after adoptive transfer of H-2^b secondaries; (*D*) persistently infected, nontransgenic mouse, day 15 after adoptive transfer of H-2^b secondaries; (*E*) persistently infected, NSE-D^b transgenic mouse, day 40 after adoptive transfer of H-2^b secondaries. ×100.

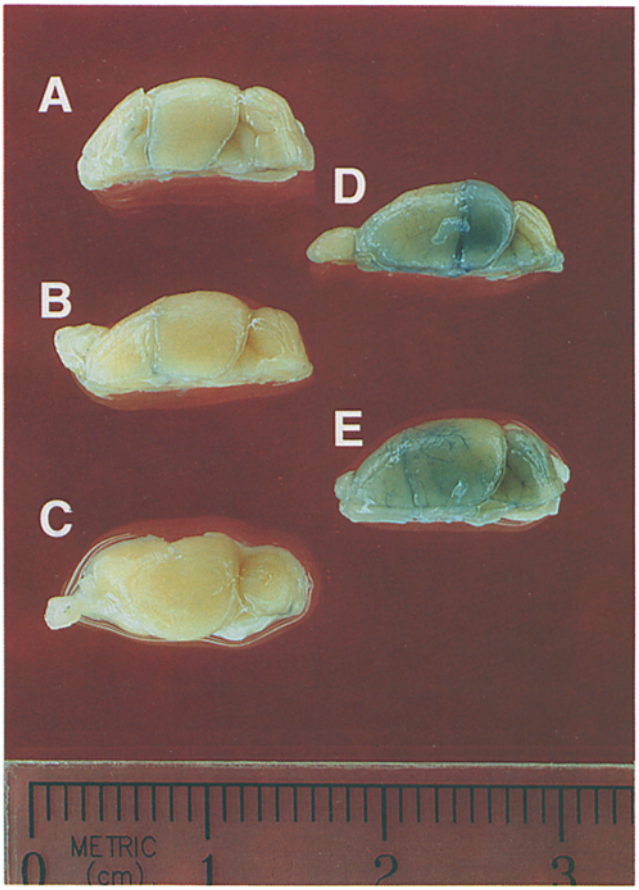

Figure 8. Abnormal permeability of the blood–brain barrier in persistently infected NSE-D^b mice after adoptive transfer of H-2^b LCMV-specific CTL. Persistently infected mice were injected with Evans blue and perfused as described in Materials and Methods. (*A*) Nontransgenic mouse, no CTL given; (*B*) persistently infected NSE-D^b transgenic mouse given H-2^s antiviral CTL 120 d previously; (*C*) persistently infected, nontransgenic mouse given H-2^b CTL and showing signs of sickness after 15 d after transfer; (*D* and *E*) persistently infected NSE-D^b mice given H-2^b CTL 120 d previously.

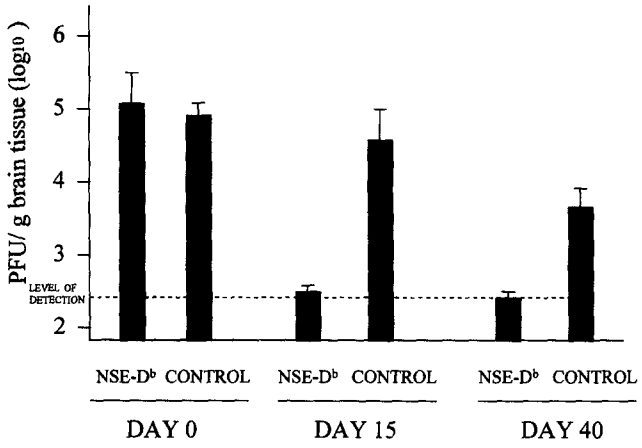

Figure 7. Clearance of infectious virus from brains of transgenic and control mice. Brains of persistently infected NSE-D^b or control (nontransgenic or NSE-APP) mice were removed 15 or 40 d after adoptive transfer of H-2^b CTL, weighed, homogenized, and analyzed for the presence of infectious virus by plaque assay. At least four animals per group were evaluated. Results represent means ± SD.

would be associated with an alteration of the blood–brain barrier, mice were injected with Evans blue, a dye which binds to serum proteins and is excluded from the brain parenchyma by the normal blood–brain barrier. In persistently infected NSE-D^b transgenic mice, leakage of the dye into the brain parenchyma was observed as early as 15 d (n = 4) (data not shown) and as late as 120 d (n = 5) after adoptive transfer of H-2^b restricted CTL (Fig. 8; Table 3). These results indicate a prolonged abnormal permeability of the blood–brain barrier, which persisted in the absence of viral antigen. Subtle differences in the pattern of permeability changes could be related to variations in the distribution or activity of the infiltrating lymphocytes.

By contrast, no other group of mice, including transiently sick nontransgenic mice killed 9 d after CTL administration, showed any abnormality in the permeability of the blood–brain barrier (Table 3; Fig. 8).

Discussion

The current study revealed a number of novel findings. The expression of class I MHC glycoproteins in neurons of NSE-D^b transgenic mice had no deleterious effects on the health, fecundity, or life span of uninfected mice. MHC class I–expressing hippocampal neurons of transgenic mice presented exogenous viral peptide to H-2–matched CTL in vitro, resulting in neuronal lysis. In vivo interactions of antiviral H-2^b–restricted CTL with D^b-expressing neurons in mice persistently infected with LCMV resulted in significant morbidity and mortality. Disease induced in the transgenic mice was associated with an increase in the number of $CD8^+$ T lymphocytes within the brain parenchyma, an increased rate of clearance of infectious virus from infected neurons, and an increased permeability of the blood–brain barrier that continued for several months after the adoptive transfer of CTL and after the virus was cleared from infected neurons. Although virus was cleared from D^b-expressing neurons by 15 d after adoptive transfer, no obvious lysis of neuronal cells was observed.

Our in vitro findings in transgenic primary embryonic neurons confirm and extend previous results obtained in OBL-21 neuronal cells (14, 17). In those studies it was shown that OBL-21 cells were unable to present viral antigen to CTL because of a paucity of class I MHC on the OBL-21 cell surface. Molecular complementation with a retrovirally expressed class I MHC molecule made infected OBL-21 cells recognizable and lysable by antiviral CTL. Although OBL-21 cells share many characteristics with neuronal cells (e.g., expression of neurofilaments, NSE, and voltage-dependent potassium channels; 22), they, unlike primary neurons, are dividing, transformed cells that grow in the presence of serum.

Expressing D^b MHC class I proteins in neuronal cells via transgenic technology and panning with mAb to D^b in the current study allowed the in vitro analysis of primary neuronal cell–virus–CTL interactions. As these primary neurons do not divide in culture and are grown in the absence of serum, they resemble differentiated neurons in the intact CNS more closely than OBL-21 cells. When transgenic D^b-expressing primary neurons were pulsed with viral peptide or infected with virus (Rall, G., and M. B. A. Oldstone, unpublished results) and challenged with MHC-matched antiviral CTL, they were recognized and lysed, whereas primary neurons from nontransgenic mice under similar conditions were not. Thus, expression of a class I MHC molecule on primary neurons in vitro was sufficient to result in lysis by appropriate CTL.

Interestingly, we did not find evidence of CTL-mediated lysis of neurons in brains of transgenic mice, despite the presence of intraparenchymal CTL and rapid viral clearance from the transgenic CNS. However, it should be noted that this study was not designed to accurately quantitate neuronal counts and that a subtle dropout of neurons may have occurred. Nevertheless, neurons of the hippocampus and Purkinje cell layer of the cerebellum are uniformly infected by LCMV in vivo (41, 42); yet at no time after adoptive transfer, in no animal, regardless of health, were morphological changes consistent with cell lysis observed in these regions. Thus, while transgenic neurons expressing the correct viral peptide bound to a transgenic MHC molecule were suitable CTL targets in vitro, such neurons did not appear to be effectively lysed in vivo. This observation may be due to a restricted number of $CD8^+$ CTL within the brain, which limits the cytotoxic effect of these cells. We believe this possibility is unlikely, however, given the rapid rate of viral clearance and blood–brain barrier damage within transgenic brains.

Alternatively, one might consider that, while these neurons express the transgene in vitro, they may not in vivo, as no direct evidence (e.g., immunohistochemical identification of the transgene product in brain sections) is provided. We feel that our inability to detect the class I molecule in brain sections was due to technical problems regarding the difficulty of immunostaining within the brain, coupled with the lack of a suitable, high-affinity antibody, and not due to the absence of expression in vivo. The adoptive transfer experiments provide strong evidence for functional protein expression in vivo: three groups of mice (NSE-D^b, NSE-APP, and nontransgenic littermates), two lines of the NSE-D^b mice (lines 9 and 11), and two different immune lymphocyte haplotypes (b and s) were used to demonstrate a selective effect of H-2^b antiviral lymphocytes on virally infected H-2^b transgenic neuronal targets. Because only the combination of mice expressing the D^b molecule with MHC-matched H-2^b immune lymphocytes resulted in pathological conditions (increased intraparenchymal CTL, increased blood–brain barrier permeability, prolonged sickness), functional expression of the class I MHC transgene in neurons is the most plausible explanation for these data.

How these CTL exert their virucidal, but not cytocidal, effects in vivo is not known. It is conceivable that factors released from neurons or glial cells interfere with lytic CTL–neuronal interactions contributing to the relatively immunosuppressive/immune-privileged microenvironment of the CNS, and that such factors interfere with the CTL-mediated cytolysis of virally infected neurons. Isolation of

Table 2. *Accumulation of CD8$^+$ T Cells Present in Brains of Mice Receiving Adoptive Transfer of Immune Lymphocytes**

Group	Mice	Virus	Transgene	Adoptive transfer of H-2^b anti-LCMV CTL (day observed)	Median number of intraparenchymal CD8$^+$ T cells‡	
					Median	Range
	n					
A	3	−	+	−	12	(0–17)
B	4	+	−	−	48	(19–82)
C	4	+	+	−	55	(14–89)
D	5	+	+	+ (15)	124	(48–211)
E	4	+	−	+ (15)	62	(23–77)
F	4	+	+	+ (40)	51	(15–69)

*Persistently infected (groups B–F) or uninfected (group A) NSE-D^b transgenic (groups A, C, D, and F) or nontransgenic (groups B and E) mice were given either H-2^b–restricted antiviral CTL (groups D–F) or no CTL (groups A–C). Brains were examined immunohistochemically for the presence of CD8$^+$ T cells at either 15 (groups D and E) or 40 (group F) d after transfer as described in Materials and Methods.
‡Values given represent the median number of CD8$^+$ positive cells found/horizontal brain section (determined from four sections per mouse, three–five mice per group).

Table 3. *Increase in Transgenic Mouse Blood–Brain Barrier Permeability Is Dictated by Expression of the Transgene in Virally Infected Mice Given Haplotype-matched Antiviral CTL**

Experimental model			Result
Neurons expressing in vivo		Adoptive transfer of D^b-restricted, LCMV- specific CTL	Percent incident of increased blood–brain barrier permeability
MHC class I	LCMV		
+	+	+	100 (5/5)
+	−	−	0 (0/5)
+	−	+	0 (0/3)
+	+	−	0 (0/4)
−	+	+	0 (0/5)
−	+	−	0 (0/5)

*Persistently infected (+) or uninfected (−) NSE-D^b transgenic (+) or nontransgenic (−) mice were given D^b-restricted antiviral CTL 120 d before blood–brain barrier assessment. Permeability was determined by entry of Evans blue into the parenchyma. The percentages of mice with Evans blue infiltration are given, with the total number of mice per group in parentheses.

neurons from this environment by in vitro culture may remove them from these factors and allow lysis to occur.

Expression of a functional MHC molecule on neurons also induced a protracted disruption of the blood–brain barrier. This effect may be mediated by soluble CTL products such as cytokines. Since the effector cells in the adoptive splenocyte transfer have been shown to be virus-specific CD8$^+$ (41), the most likely cytokines would be IFN-γ and TNF-α. Studies to directly test this hypothesis are currently underway using IFN-γ and TNF-α knockout mice. Cytokines have recently been shown to cause direct injury within the CNS in which injection (45) or endogenous synthesis (46) of cytokines resulted in several clinical and histopathologic changes within the brain, including blood–brain barrier damage.

After adoptive transfer of MHC-restricted virus-specific CTL into mice whose neurons contain virus but lack expression of MHC class I molecules, clearance occurs by 60–120 d (41). Interestingly, in the model reported here, clearance occurs much earlier, by day 15 (Fig. 7). In both instances, clearance of virus from neurons occurs in the absence of lysis of these cells, suggesting that intraparenchymal cytokine production may also participate in the clearance of infectious virus from transgenic brains. Inhibition of viral synthesis in the absence of cell death has been documented in the CTL response to hepatitis B virus infection of hepatocytes (47–49) and in HIV (50). In these systems, inflammatory cytokines such as IFN-γ and TNF-α can

down-regulate viral expression without concomitant cell death.

The stability of the blood–brain barrier is critical to the exclusion of potentially neurotoxic or cytolytic blood-borne molecules (such as cytokines) from the CNS parenchyma as well as to the maintenance of an appropriate homeostatic osmotic balance within the brain (51). While rapid alterations in the permeability of the blood–brain barrier may result in edema and death (52, 53), slower changes in permeability need not be life threatening but may lead to more chronic neurologic symptoms (54). In agreement with this concept, we observed a significant increase in animal sickness and death at early times after adoptive transfer, with continued sickness of surviving mice throughout the 150-d observation period.

Several CNS diseases have been associated with transient or permanent changes in blood–brain barrier permeability, including those associated with CNS infection (54–58), demyelinating diseases (59), and tumors (60). Our study, in which abnormal permeability of the blood–brain barrier and clinical illness were detected long after virus had been cleared from the mice (Fig. 7), indicates that viral infections and CTL–CNS interactions may induce blood–brain barrier disruptions and neurologic disease by a "hit-and-run" mechanism, triggering a cascade of pathogenic events that proceeds in the absence of continual viral stimulation.

The authors acknowledge the technical assistance of Monica Hambalko and Liliana Riquer, the secretarial aid of Amy Goddard, and David Bloom for reviewing the manuscript.

This work was supported by U.S. Public Health grants AI09484 and NS12428, and by a fellowship from the J. D. and Ida Leiper Foundation and National Institutes of Health training grant MH19185 to G. F. Rall. L. Mucke was supported, in part, by a Harry Weaver Neuroscience Scholarship Award from the National Multiple Sclerosis Society.

Address correspondence to Glenn F. Rall, The Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

Received for publication 2 February 1995 and in revised form 31 May 1995.

References

1. Medawar, P. 1974. Transplantation biology: an appraisal. *Mt. Sinai J. Med.* 41:760–764.
2. Bartlett, P.F., R.S.C. Kerr, and K.A Bailey. 1989. Expression of major histocompatibility antigens in the central nervous system. *Transplant. Proc.* 21:3163–3165.
3. Greenwood, J. 1991. Mechanisms of blood-brain barrier breakdown. *Neuroradiology.* 33:95–100.
4. Streilein, J.W. 1993. Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. *Curr. Opin. Immunol.* 5:428–432.
5. Wekerle, H., C. Linington, H. Lassmann, and R. Meyermann. 1986. Cellular immune reactivity within the CNS. *Trends Neurosci.* 9:271–277.
6. Wekerle, H., D. Sun, R.L. Oropeza-Wekerle, and R. Meyermann. 1987. Immune reactivity in the nervous system: modulation of T lymphocyte activation by glial cells. *J. Exp. Biol.* 132:43–57.
7. Hickey, W.F., and H. Kimura. 1987. Graft versus host disease elicits expression of class I and II MHC antigens and presence of scattered T lymphocytes in the rat CNS. *Proc. Natl. Acad. Sci. USA.* 84:2082–2086.
8. Hickey, W.F., B.L. Hsu, and H. Kimura. 1991. T lymphocyte entry into the central nervous system. *J. Neurosci. Res.* 28:254–260.
9. Oldstone, M.B.A., and P.J. Southern. 1993. Trafficking of activated CTL into the central nervous system: use of a transgenic model. *J. Neuroimmunol.* 46:25–31.
10. Lampson, L. 1987. Molecular bases of the immune response to neural antigens. *Trends Neurosci.* 10:211–216.
11. Drew, P., M. Lonergan, M. Goldstein, L. Lampson, K. Ozato, and D. McFarlin. 1993. Regulation of MHC class I and β_2-microglobulin gene expression in human neuronal cells. *J. Immunol.* 150:3300–3310.
12. Massa, P.T., K. Ozato, and D.E. McFarlin. 1993. Cell type specific regulation of major histocompatibility complex (MHC) class I gene expression in astrocytes, oligodendrocytes and neurons. *GLIA.* 8:201–207.
13. Lawrence, J.M., R.J. Morris, D.J. Wilson, and G. Raisman. 1990. Mechanisms of allograft rejection in the rat brain. *Neuroscience.* 37:431–462.
14. Joly, E., L. Mucke, and M.B.A. Oldstone. 1991. Viral persistence in neurons explained by a lack of MHC class I expression. *Science (Wash. DC).* 253:1283–1285.
15. Mucke, L., and M.B.A. Oldstone. 1992. The expression of MHC class I antigens in the brain differs markedly in acute and persistent infections with lymphocytic choriomeningitis virus. *J. Neuroimmunol.* 36:193–198.
16. Lampson, L., and C. Fisher. 1984. Weak HLA and β-2 microglobulin expression of neuronal cell lines can be modulated by interferon. *Proc. Natl. Acad. Sci. USA.* 81:6476–6480.
17. Joly, E., and M.B.A. Oldstone. 1992. Neuronal cells are deficient in loading peptides onto MHC class I molecules. *Neuron.* 8:1185–1190.
18. Keane, R.W., M.W. Tallent, and E.R. Podack. 1992. Resistance and susceptibility of neural cells to lysis by cytotoxic lymphocytes and by cytolytic granules. *Transplantation (Baltimore).* 54:520–526.

19. Schlitt, M., R.B. Chronister, and R.J. Whitley. 1991. Pathogenesis and pathophysiology of viral infections of the central nervous system. *In* Infections of the Central Nervous System. W. Scheld, editor. Raven Press, New York. 7–18.
20. Ahmed, R., and J.G. Stevens. 1990. Viral persistence, *In* Field's Virology. B.N. Fields and D.M. Knipe, editors. Raven Press, New York. 241–266.
21. Esiri, M.M., and P.G.E. Kennedy. 1992. Virus diseases, *In* Greenfield's Neuropathology. Fifth Ed. J.H. Adams and L.W. Duchen, editors. Oxford University Press, New York. 335–399.
22. Ryder, E.F., E.Y. Snyder, and C.L. Cepko. 1990. Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. *J. Neurobiol.* 21:356–375.
23. Dutko, F.J., and M.B.A. Oldstone. 1983. Genomic and biological variation among commonly used lymphocytic choriomeningitis virus strains. *J. Gen. Virol.* 64:1689–1698.
24. Oldstone, M.B.A., R. Ahmed, M.J. Buchmeier, P. Blount, and A. Tishon. 1985. Perturbation of differentiated functions during viral infections in vivo. I. Relationship of lymphocytic choriomeningitis virus and host strains to growth hormone deficiency. *Virology.* 142:158–174.
25. Whitton, J.L., J.R. Gebhard, H. Lewicki, A. Tishon, and M.B.A. Oldstone. 1988. Molecular definition of a major cytotoxic T lymphocyte epitope in the glycoprotein of lymphocytic choriomeningitis virus. *J. Virol.* 62:687–695.
26. Whitton, J.L., A. Tishon, H. Lewicki, J. Gebhard, T. Cook, M. Salvato, E. Joly, and M.B.A. Oldstone. 1989. Molecular analyses of a five amino acid cytotoxic T lymphocyte (CTL) epitope: an immunodominant region which induces nonreciprocal CTL cross reactivity. *J. Virol.* 63:4303–4310.
27. Buchmeier, M.J., R.M. Welsh, F.J. Dutko, and M.B.A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* 30: 275–331.
28. Ausubel, F.M., R. Brent, and R.E. Kingston. 1987. Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley Interscience, New York, NY. 2.0.1–3.19.8.
29. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
30. Sakimura, K., E. Kushiya, Y. Takahashi, and Y. Suzuki. 1987. The structure and expression of the neuron-specific enolase gene. *Gene (Amst.).* 60:103–113.
31. Abraham, C.R., K. Kanemuru, and L. Mucke. 1993. Expression of cathepsin-G-like and alpha-1 antichymotrypsinlike proteins in reactive astrocytes. *Brain Res.* 621:222–232.
32. Allen, H., J. Fraser, D. Flyer, S. Calvin, and R.A. Flavell. 1986. β-2-microglobulin is not required for cell surface expression of the murine class I histocompatibility antigen $H\text{-}2D^b$ or of a truncated $H\text{-}2D^b$. *Proc. Natl. Acad. Sci. USA.* 82:7447–7451.
33. Hogan, B., F. Constantini, and E. Lacy. 1986. Manipulating the Mouse Genome, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
34. Mucke, L., M.B.A. Oldstone, J.C. Morris, and M. Nerenberg. 1991. Rapid activation of astrocyte-specific expression of GFAP-lacZ transgene by focal injury. *New Biol.* 3:465–474.
35. Oldstone, M.B.A., M.I. Nerenberg, P.J. Southern, J. Price, and H. Lewicki. 1991. Virus infection triggers insulin dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell.* 65:319–331.
36. Banker, G., and K. Goslin. 1991. Rat hippocampal neurons in low density culture. *In* Culturing Nerve Cells. G. Banker and K. Goslin, editors. The MIT Press, Cambridge, MA. 251–281.
37. Pasick, J.M.M., K. Kalicharran, and S. Dales. 1994. Distribution and trafficking of JHM coronavirus structural proteins and virions in primary neurons and the OBL-21 neuronal cell line. *J. Virol.* 68:2915–2928.
38. MacLeish, P.R., C.J. Barnstable, and E. Townes-Anderson. 1983. Use of a monoclonal antibody as a substrate for mature neurons in vitro. *Proc. Natl. Acad. Sci. USA.* 80:7014–7018.
39. O'Malley, M.B., and P.R. MacLeish. 1993. Induction of class I major histocompatibility complex antigens on adult primate retinal neurons. *J. Neuroimmunol.* 43:45–58.
40. Rall, G.F., L. Mucke, M. Nerenberg, and M.B.A. Oldstone. 1994. A transgenic mouse model to assess the interaction of cytotoxic T lymphocytes with virally infected, class I MHC-expressing astrocytes. *J. Neuroimmunol.* 52:41–50.
41. Oldstone, M.B.A., P. Blount, P.J. Southern, and P.W. Lampert. 1986. Cytoimmunotherapy for persistent viral infection reveals a unique clearance pattern from the central nervous system. *Nature (Lond.).* 321:239–243.
42. Rodriguez, M., M.J. Buchmeier, M.B.A. Oldstone, and P.W. Lampert. 1983. Ultrastructural localization of viral antigens in the central nervous system of mice persistently infected with LCMV. *Am. J. Pathol.* 110:95–100.
43. Fazakerley, J.K., P.J. Southern, F.E. Bloom, and M.J. Buchmeier. 1991. High resolution in situ hybridization to determine the cellular distribution of LCMV RNA in the tissues of persistently infected mice: relevance to arenavirus disease and mechanisms of viral persistence. *J. Gen. Virol.* 72:1611–1625.
44. Tishon, A., M. Eddelston, J.C. de la Torre, and M.B.A. Oldstone. 1993. Cytotoxic T lymphocytes cleanse viral gene products from individually infected neurons and lymphocytes in mice persistently infected with LCMV. *Virology.* 197:463–467.
45. Watts, R.G., J.L. Wright, L.L. Atkinson, and R.E. Merchant. 1989. Histopathological and blood brain barrier changes in rats induced by an intracerebral injection of human recombinant interleukin-2. *Neurosurgery (Baltimore).* 25:202–208.
46. Campbell, I.L., C.R. Abraham, E. Masliah, P. Kemper, J.D. Inglis, M.B.A. Oldstone, and L. Mucke. 1993. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin-6. *Proc. Natl. Acad. Sci. USA.* 90:10061–10065.
47. Gilles, P.N., G. Fey, and F.V. Chisari. 1992. Tumor necrosis factor alpha negatively regulates hepatitis B virus gene expression in transgenic mice. *J. Virol.* 66:3955–3960.
48. Guilhot, S., L.G. Guidotti, and F.V. Chisari. 1993. Interleukin-2 downregulates hepatitis B virus gene expression in transgenic mice by a posttranscriptional mechanism. *J. Virol.* 67:7444–7449.
49. Guidotti, L.G., K. Ando, M.V. Hobbs, T. Ishikawa, L. Runkel, R.D. Schreiber, and F.V. Chisari. 1994. Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. *Proc. Natl. Acad. Sci. USA.* 91:3764–3768.
50. Hseuh, F., C. Walker, D. Blackbourn, and J. Levy. 1994. Suppression of HIV replication by $CD8^+$ cell clones derived from HIV-infected and uninfected individuals. *Cell. Immunol.* 159:271–279.

51. Rapoport, S.I. 1976. Blood-Brain Barrier in Physiology and Medicine. Raven Press, New York. 316 pp.
52. Pappius, H.M., and W. Feindel. 1976. Dynamic Aspects of Brain Edema. Springer, Heidelberg and Berlin. 18–22.
53. Miller, J.D., and J.H. Adams. 1992. The pathophysiology of raised intracranial pressure. *In* Greenfield's Neuropathology. Fifth ed. J.H. Adams and L.W. Duchen, editors. Oxford University Press, New York. 69–105.
54. Power, C., P.A. Kong, T.O. Crawford, S. Wesselingh, J.D. Glass, J.C. McArthur, and B.D. Trapp. 1993. Cerebral white matter changes in AIDS dementia: alterations of the blood brain barrier. *Ann. Neurol.* 34:339–350.
55. Chaturvedi, U.C., R. Dhawan, M. Khanna, and A. Mathur. 1991. Breakdown of the blood brain barrier during Dengue virus infection of mice. *J. Gen. Virol.* 72:859–866.
56. Mathur, A., N. Khanna, and U.C. Chaturvedi. 1992. Breakdown of blood brain barrier by virus-induced cytokines during Japanese encephalitis virus infection. *Int. J. Exp. Pathol.* 73:603–611.
57. Anderson, I.H., O. Marker, and A.R. Thomsen. 1991. Breakdown of blood brain barrier function in the murine LCMV infection mediated by virus-specific $CD8^+$ T cells. *J. Neuroimmunol.* 31:155–163.
58. Soilu-Hanninen, M., J.P. Earlinna, V. Hukkanen, M. Roytta, A.A. Salmi, and R. Salonen. 1994. Semliki forest virus infects mouse brain endothelial cells and causes blood-brain barrier damage. *J. Virol.* 68:6291–6298.
59. Sharief, M.K., M.A. Noori, M. Ciardi, A. Cirelli, and E.J. Thompson. 1993. Increased levels of circulating ICAM-1 in serum and CSF of patients with active multiple sclerosis: correlation with TNF alpha and blood brain barrier damage. *J. Neuroimmunol.* 43:15–22.
60. Adams, J.H., and L.W. Duchen, editors. 1992. Greenfield's Neuropathology. Fifth ed. Oxford University Press, New York. 1557 pp.